DTIC FILE COPY

EPORT DOCUMENTATION PAGE

AD-A223 526

1b. RESTRICTIVE MARKINGS

3. DISTRIBUTION / AVAILABILITY OF REPORT
Approved for public release; distribution is unlimited

2b. DECLASSIFICATION / DOWNGRADING SCHEDULE

4. PERFORMING ORGANIZATION REPORT NUMBER(S)
NMRI 90-44

5. MONITORING ORGANIZATION REPORT NUMBER(S)

6a. NAME OF PERFORMING ORGANIZATION
Naval Medical Research

6b. OFFICE SYMBOL (If applicable)

7a. NAME OF MONITORING ORGANIZATION
Naval Medical Command

6c. ADDRESS (City, State, and ZIP Code)
Bethesda, Maryland 20814-5055

7b. ADDRESS (City, State, and ZIP Code)
Department of the Navy
Washington, D.C. 20372-5120

8a. NAME OF FUNDING / SPONSORING ORGANIZATION
Naval Medical Research and Development Command

8b. OFFICE SYMBOL (If applicable)

9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER

8c. ADDRESS (City, State, and ZIP Code)
Bethesda, Maryland 20814-5055

10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO
63706N	M0096.004	0006	DN377025

11. TITLE (Include Security Classification)
The Determination of the Repeated Oral Toxicity of Halocarbon Oil, Series 27-S.

12. PERSONAL AUTHOR(S) Kinkead ER, Culpepper BT, Henry SS, Szotak PS, Flemming CD, Kutzman RS Bruner RH, Wyman JF, Mattie DR

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
journal article	FROM _____ TO _____	1990	16

16. SUPPLEMENTARY NOTATION
Reprinted from: Toxicology and Industrial Health, Vol. 6, No. 1, 1990, pp. 17-32

17. COSATI CODES		
FIELD	GROUP	SUB-GROUP

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
Bone, Calcium, Fluoride, Phosphates, Rats

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

20. DISTRIBUTION / AVAILABILITY OF ABSTRACT
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS

21. ABSTRACT SECURITY CLASSIFICATION
Unclassified

22a. NAME OF RESPONSIBLE INDIVIDUAL
Phyllis Blum, Information Services Division

22b. TELEPHONE (Include Area Code)
202-295-2188

22c. OFFICE SYMBOL
ISD/ADMIN/NMRI

THE DETERMINATION OF THE REPEATED ORAL TOXICITY OF HALOCARBON OIL, SERIES 27-S

EDWIN R. KINKEAD*, BRENDA T. CULPEPPER*, SANDRA S. HENRY*, PHYLLIS S. SZOTAK*, CARLYLE D. FLEMMING*, RAYMOND S. KUTZMAN*, RICHARD H. BRUNER†, JOHN F. WYMAN† AND DAVID R. MATTIE‡

Halocarbon 27-S (HC 27-S), a polymer of chlorotrifluoroethylene (CTFE), is used as a lubricating oil for pumps in hyperbaric chambers. Although monomeric CTFE has been shown to produce renal lesions in rats, the toxicity of CTFE polymers have not been investigated. To assess the toxicity of repeated exposure to HC 27-S, three groups (N = 6/group) of male and female Fischer-344 rats were dosed with 2.5 g HC 27-S/kg for 7 or 21 consecutive days. Groups were sacrificed at 7, 21, and 35 days (14 days after the 21-day dosing). Corresponding control groups (N = 6) were dosed with deionized water. Decreased water consumption and urine output were apparent in all test groups. Statistically significant increases in fluoride excretion were noted in 24-hr urine samples assessed periodically during the study. Neurotoxic signs were observed in female rats but not in male rats. Significant increases in liver and kidney weights were seen in all rats, regardless of number of dosing days. The increased fluoride burden in treated animals appeared sufficient to alter bone calcium/phosphate ratios in male rats but not female rats. Gross liver enlargement and hepatocellular cytomegaly indicated that the liver was probably the primary target organ following repeated administration of HC 27-S. *(Supported by US Navy through Air Force Contract # F33615-085-C-0532.)*

* NSI Technology Services Corporation, 101 Woodman Drive, Suite 12, Dayton, Ohio 45431.
† Naval Medical Research Institute/Toxicology Detachment, Wright-Patterson AFB, Ohio 45433.
‡ Harry G. Armstrong Aerospace Medical Research Laboratory, Wright-Patterson AFB, Ohio 45433.
1. Address correspondence to: Mr. Edwin R. Kinkead, NSI Technology Services Corp., 101 Woodman Drive, Suite 12, Dayton, Ohio 45431.
2. Key words: oral, halocarbon oil, hyperbaric chambers, fluoride.
3. Abbreviations: BUN, CAP, CTFE, F-344, HC 27-S, LDH, SEM.

Toxicology and Industrial Health, 6:1, pp. 17-32

ISSN: 0748-2337

INTRODUCTION

Halocarbon Oil, Series 27-S (HC 27-S) is a polymer of chlorotrifluoroethylene (CTFE) and is used as a lubricating oil for pumps employed in hyperbaric chambers by the US Navy Sea Systems Command. In addition, HC 27-S is used to lubricate all O-rings on doors and service locks of the hyperbaric chambers. Although no specific toxicity information is available on the polymers of CTFE, monomeric CTFE has been shown to produce renal damage in rats (Potter et al., 1981; Buckley et al., 1982). The principal insult in the kidney was necrosis of the *pars recta*, and to a lesser extent, the *pars convoluta* of the proximal tubule. Signs of nephrotoxicity included diuresis, increased urinary lactic dehydrogenase (LDH) activity, serum creatinine, and blood urea nitrogen (BUN) with a concurrent decrease in urine osmolality. Water intake was increased by 25% in exposed rats (Potter et al., 1981). CTFE is metabolized to inorganic fluoride which is excreted in the urine. Plasma and urine fluoride levels in Fischer-344 (F-344) rats remained elevated for more than a week following oral exposure and for at least 24 hr following inhalation exposure to CTFE. Neither plasma nor urine fluoride levels were elevated following dermal exposure (Kinkead et al., 1987). In subchronic studies, renal tubules underwent regeneration and necrosis was minimal upon further exposure, suggesting adaptation to CTFE toxicity (Buckley et al., 1982). The authors speculated that metabolism and/or disposition of CTFE was altered, or that regenerated tissue was refractive to CTFE toxicity.

The rat was selected as the current test species to minimize space requirements and to allow comparison to the above mentioned CTFE studies. The numbers of animals per test group were kept to the minimum necessary for appropriate statistical analysis. These studies were performed as a preliminary assessment of the toxicity of repeated exposure to HC 27-S.

MATERIALS AND METHODS

Test Material

The HC 27-S test material was supplied by the Naval Medical Research Institute/Toxicology Detachment at Wright-Patterson Air Force Base. It was manufactured by Halocarbon Products Corporation (Hackensack, NJ) to conform to US Military Specification MIL-L-24574. The oil has a vapor pressure of less than 0.01 mm Hg at 27°C and contains 0.1% of an unspecified organic acid rust inhibitor. This sample had a density of 1.930 g/ml at room temperature and decomposes at temperatures above 260°.

Animals

Fischer-344 rats, males weighing between 180 and 220 g and females between 150 and 220 g, were purchased from Charles River Breeding Labs of Kingston, NY. Quality control assessments, conducted during a two-week quarantine period, showed the animals to be in acceptable health. The animals used in this study were handled in accordance with the principles stated in the *Guide for the Care and Use of Laboratory Ani-*

mals, prepared by the Committee on Care and Uses of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, DHHA, National Institute of Health Publication #85-23, 1985, and the Animal Welfare Act of 1966, as amended.

The rats were individually housed except during the quarantine period and the 14-day holding period postdosing. Prior to treatment, the rats were put into Nalgene® (Nalge Company, Rochester, NY) metabolism cages for a one-week acclimatization period. All rats were fed Purina Formulab 5008® (food in metabolism cages was powdered) and deionized distilled water *ad libitum*, with the exception that rats were fasted for 16 hr prior to oral dosing. All animals were identified by toe clipping and were maintained on a light/dark cycle set at 12 hr intervals. The test material was administered orally by gavage at a volume of 10 ml/kg body weight using a ball-tipped, straight, stainless-steel gavage needle. Dosing was performed between 8 and 9 AM each day.

Repeated Oral Assay

The study was designed to involve repeated dosing at one-half the acute oral LD_{50} value. However, because no deaths occurred at the acute limit test of 5 g/kg (Kinkead et al., 1989) the daily treatment was set at 2.5 g HC 27-S/kg. Six groups of six F-344 rats each (per sex) were dosed daily, including weekends, by gavage. Each of three test groups per sex received the same daily dose but the groups were sacrificed at different times. Each of three control groups received an equal volume of distilled water and were sacrificed with their corresponding treatment groups. The HC 27-S was administered as neat agent and the dose volumes were calculated from the individual body weights, adjusted daily. Dosing was performed at the beginning of each day, prior to 0930 hr. Food, provided following gavage, was removed at 1630 hr each day.

Body weights were measured daily throughout the study. Water consumption and urine output were gravimetrically measured daily during the dosing period. Urine samples from one day predosing, from days 1, 3, 5, 7, 14, and 21 during dosing, and from day 14 postdosing were clinically analyzed. Urinary measurements (Ames Multistix, Ames Division, Miles Laboratories, Elkhart, IN) included pH, protein, bilirubin, urobilinogen, glucose, ketone, and occult blood. In addition, specific gravity and creatinine values were obtained.

The rats were fasted sixteen hr prior to sacrifice. To eliminate the possible chemical interference of halocarbon anesthesia, carbon dioxide inhalation was used for euthanasia. One test and one control group were sacrificed on the morning following the seventh day of dosing; a second group of test and control rats were sacrificed on the morning following the 21st day of dosing; and the final groups (one test and one control) were sacrificed 14 days after the completion of dosing. At the time of sacrifice, blood was collected from the posterior *vena cava* for whole blood and serum analyses. The following organs were weighed: heart, pituitary, liver, spleen, thymus, kidneys, testes, ovaries, and brain. A gross pathologic examination was performed on each rat and

specified tissues were collected and prepared for histopathologic examination. The right femur of each was collected for x-ray elemental analysis and scanning electron microscopy (SEM).

Fluoride Analysis

A fluoride specific ion electrode as described by Neefus et al. (1970) was used to determine fluoride ion content in urine. This method utilized synthetic urine as well as a buffer for standardization. The method of Singer and Ophaug (1979) was used to determine unbound or ionic fluoride in plasma. A fluoride specific electrode directly measured fluoride ion concentration following dilution of the plasma in buffer.

X-Ray Elemental Analysis

X-ray elemental analysis was conducted on the right femur of three rats of each sex per group to assess Ca and P content. The femur was cleaned, the ends removed, and fixed in 10% neutral buffered formalin for 48 hr. At midshaft, the femurs were trimmed into 7–10 mm lengths and were processed for SEM and x-ray analysis by dehydration with a graded series of alcohols (ethanol). The femurs were sealed into parafilm cylinders containing 100% alcohol, frozen in liquid nitrogen and then fractured into two halves. After thawing in fresh 100% alcohol, the femurs were dried and mounted on aluminum stubs. For SEM, the fracture face was mounted in an upright position and sputter coated with gold. The femurs were mounted on their side and a low power SEM photograph documented the site of x-ray analysis. Six spectra were collected from each femur in two sets of three. Spectra were collected from right to left in each set. The second set (D,E,F) were collected to the left of the first set (A,B,C). Spectra were analyzed for Ca and P using the software in an x-ray analysis system (Model 7700, Kevex Corp., San Carlos, CA).

Statistical Analysis

Statistical analyses of data were as follows: a repeated multivariate analysis of variance with Ryan-Einot-Gabriel-Welsh F-tests used for comparisons (Dixon, 1985: Barcikowski, 1983) of body weights, water consumption, urine volumes, and urine and blood chemistries between control and test groups; a two factorial analysis of variance was performed for both sets of blood parameters, calcium-phosphorus (CaP) ratios, and organ weights (Dixon, 1985; Barcikowski, 1983). Histopathologic data were analyzed using the Yates' corrected Chi-square test (Zar, 1974). A probability of 0.05 or less denoted a statistically significant change from controls.

RESULTS

Repeated oral dosing of HC 27-S to male and female rats resulted in unthrifty hair coat, lethargy, and diarrhea. Three test and two control male rats and four test female rats died during the repeated dosing regimen prior to scheduled sacrifice. Of these, two test and one control male rat and two of four female rats had sufficient gavage trauma as to

have been the cause of death. Tonoclonic spasms were noted in the female test rats after four days of treatment and were noted sporadically throughout the rest of the study. Convulsions were observed in four different rats on three separate occasions. Not all female rats were observed to have convulsed, nor was a single rat observed to have convulsed more than once. Test subjects of both sexes had mild diarrhea by days 5 to 6 and appeared lethargic by days 8 to 10. The female rats appeared unkempt and kyphotic by day 11. A few treated rats had dried blood and/or hematoporphyrin-like residue around the mouth and nares. Convulsions were not noted in male rats at any time throughout the study.

Both sexes of rats dosed with HC 27-S had lower mean body weights than their respective control group at most weighing dates (Figure 1). Following an early depression in the mean body weight of the male test group (days 1 and 3), no difference from the controls was noted until day 17, after which the mean body weights of the test group were depressed ($p < 0.01$) throughout the remainder of the study. The mean body weight of the female test group was generally lower than the control group but the difference was statistically significant only on days 3 through 17.

The mean daily water consumption of treated animals was depressed ($p < 0.01$) throughout the 21-day treatment period (Figure 2). Overall, the treated male rats consumed 17% less water than their respective controls while the test female animals consumed 25% less water than the controls. Commensurate with the depressed daily water consumption was a decrease in urine output in both sexes of rats. Urine output by both male and female test animals was generally depressed ($p < 0.01$) throughout the treatment period. Based on the days when urine volumes were measured, male treated animals produced 75% the urine volume of control males while the female treatment group produced only 54% the urine volume of control females.

There was a significant decrease in serum glucose values for the test male rats at each evaluation period while female test animals had increased ($p < 0.01$) glucose concentrations at 21 and 35 days (Tables 1 and 2). The sera of both sexes had increased ($p < 0.05$) albumin concentrations at 21 and 35 days but only the females had a commensurate increase in total protein at those evaluation periods. Several parameters noted as significant in male rats were outside normal ranges and appear to be treatment-related. BUN concentrations were 28 and 78% greater ($p < 0.05$) than control concentrations at 21 and 35 days, respectively. Serum concentrations of alkaline phosphatase of the treated animals progressively increased ($p < 0.01$) by 35, 179, and 216% greater than control concentrations at each of the evaluation periods. Albumin concentrations were also higher ($p < 0.05$) at each of the evaluation periods; however, the increases above control values were not as dramatic (8, 25, and 21%, respectively, at the 7-, 21-, and 35-day evaluations).

Lymphocytosis was a common finding in both sexes of test rats sacrificed at 21 and 35 days. The increase ($p < 0.05$) in the numbers of lymphocytes was commensurate with

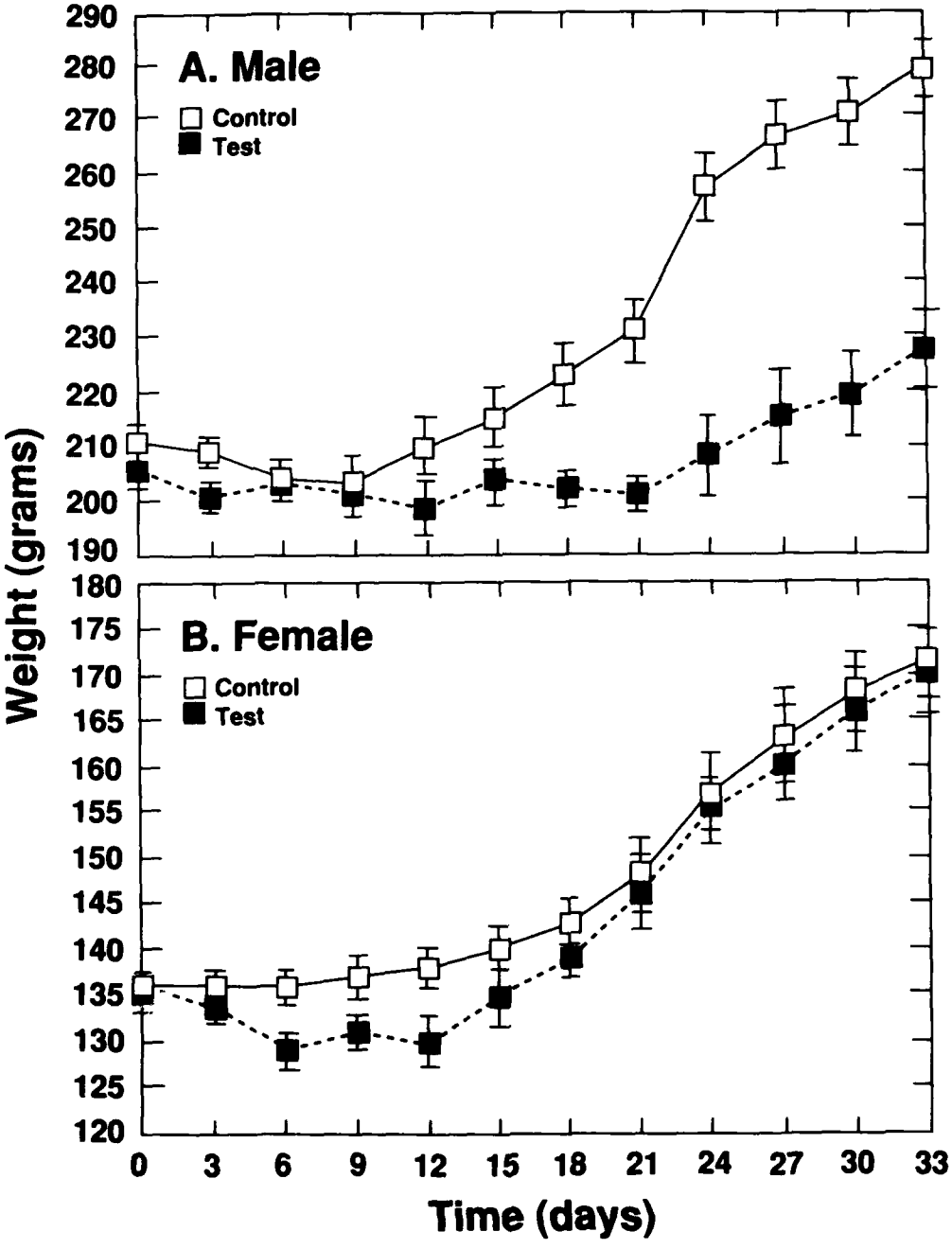

Figure 1. Mean body weights (± S.E.M.) for male and female Fischer-344 rats following repeated oral administration of Halocarbon 27-S. N = 14 to 18 for days 1 through 7; 8 to 12 for days 8 through 21; and 4 to 6 for days 21 through 35.

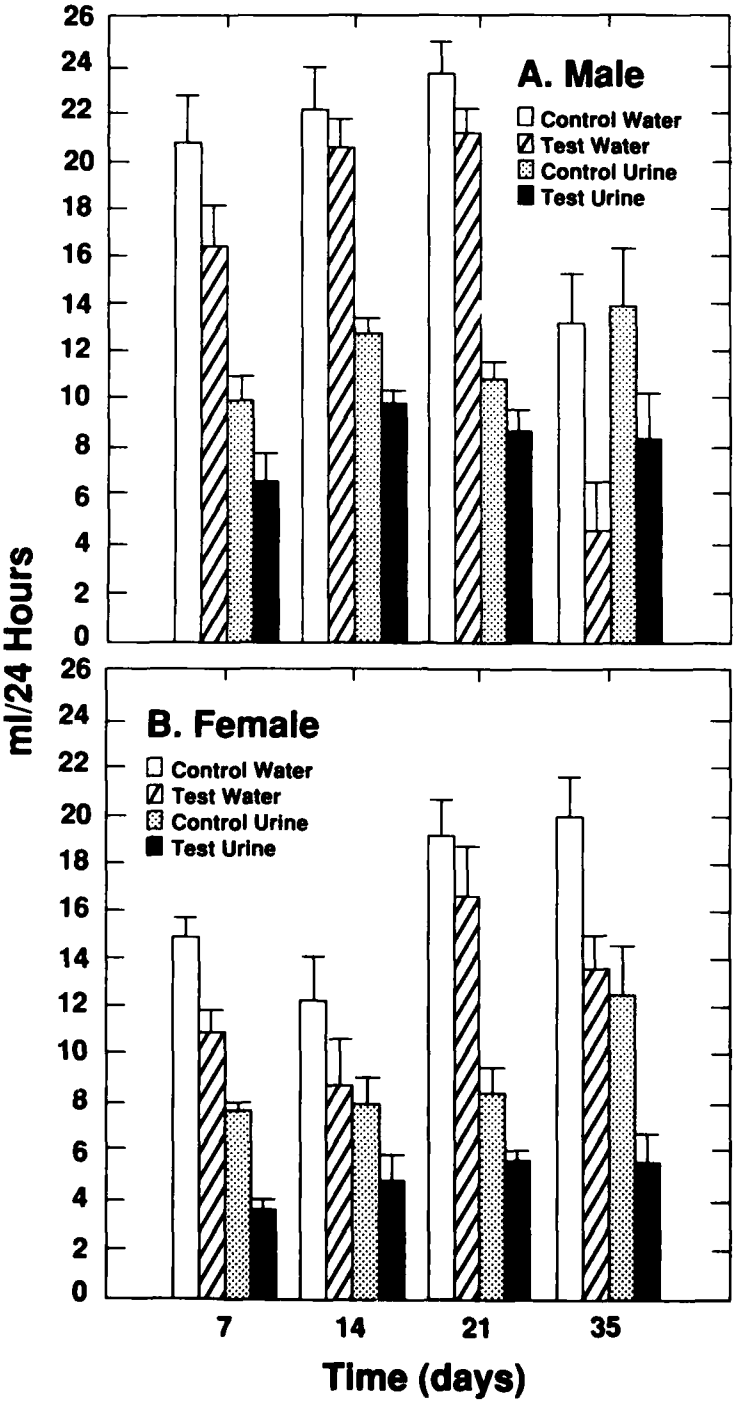

Figure 2. Water consumption versus urine output for male and female Fischer-344 rats following repeated oral administration of Halocarbon 27-S. Values are mean ± S.E.M.

TABLE 1
Serum Biochemistry Parameters[a] for Male F-344 Rats Following Repeated Oral Administration of Halocarbon 27-S

	7 Days	21 Days	35 Days
Glucose (mg/dL)			
Control	169.0 ± 14.9	207.7 ± 16.0	154.4 ± 18.9
Test	116.1 ± 9.0[b]	125.8 ± 4.7[b]	132.3 ± 5.7[b]
BUN (mg/dL)			
Control	17.9 ± 1.0	14.0 ± 0.9	16.9 ± 0.4
Test	16.5 ± 0.3	18.1 ± 1.0[c]	30.1 ± 0.7[b]
Alk. Phos. (IU/L)			
Control	115.3 ± 4.9	118.1 ± 6.3	65.3 ± 4.0
Test	155.6 ± 6.3[b]	329.3 ± 18.2[b]	206.5 ± 26.1[b]
Albumin (g/dL)			
Control	3.9 ± 0.0	3.9 ± 0.0	5.2 ± 0.1
Test	4.2 ± 0.1[c]	4.9 ± 0.1[c]	6.3 ± 0.1[c]
Total Protein (g/dL)			
Control	6.1 ± 0.1	6.3 ± 0.1	6.4 ± 0.2
Test	6.1 ± 0.1	6.9 ± 0.2	6.6 ± 0.1

[a] Mean ± S.E.M.
[b] Statistically different from controls at $p < 0.01$.
[c] Statistically different from controls at $p < 0.05$.

a similar decrease in the number of neutrophils. All other hematological parameters were similar between treated and control groups.

Inorganic fluoride concentrations of 24-h urine collections, measured periodically during the study (Figure 3), increased throughout the treatment period, especially during the first 7 days. The fluoride concentrations in the urine returned toward baseline or control levels following treatment; however, complete recovery was not achieved by 14-days posttreatment. The increase in urine fluoride concentrations was statistically significant after a single treatment in both male and female rats. Plasma inorganic fluoride concentrations of the treated rats, measured at each sacrifice, were not elevated above control values (Table 3).

X-ray elemental analysis was performed on femurs from three rats per sex per group following sacrifice to determine if deposition of fluoride in the bone matrix was sufficient to alter CaP ratios. Treated male rats had CaP ratios that were significantly greater than the ratios of the control male rats at each sacrifice period (Table 4). No CaP ratio differences were found between treated and control female rats at any of the sacrifice periods.

TABLE 2
Serum Biochemistry Parameters[a] for Female F-344 Rats Following Repeated Oral Administration of Halocarbon 27-S

	7 Days	21 Days	35 Days
Glucose (mg/dL)			
Control	71.1 ± 4.7	109.4 ± 5.3	92.2 ± 1.8
Test	79.2 ± 5.4	149.5 ± 7.7[b]	103.0 ± 7.6[b]
BUN (mg/dL)			
Control	21.9 ± 0.5	21.6 ± 0.5	15.8 ± 0.6
Test	21.7 ± 5.2	19.1 ± 1.3	16.6 ± 2.1
Alk. Phos. (IU/L)			
Control	90.9 ± 2.4	94.2 ± 3.9	73.0 ± 3.7
Test	84.3 ± 3.7	98.7 ± 4.8	129.3 ± 6.7[b]
Albumin (g/dL)			
Control	3.9 ± 0.0	3.8 ± 0.0	3.7 ± 0.0
Test	3.5 ± 0.1	4.3 ± 0.1[b]	4.4 ± 0.2[b]
Total Protein (g/dL)			
Control	6.2 ± 0.1	6.1 ± 0.1	5.8 ± 0.1
Test	5.8 ± 0.2	6.9 ± 0.2[b]	7.1 ± 0.1[b]

[a] Mean ± S.E.M.
[b] Statistically different from controls at $p < 0.01$ level.

Notable increases in relative kidney weights of the HC 27-S treated rats of both sexes occurred at each of the sacrifice periods (Table 5). The increased kidney to body weight ratio of female rats remained consistent throughout the study while the ratio in male animals continued to increase during the treatment and posttreatment period. Mean liver weights of the treated rats were markedly increased over controls except at the initial (7 day) sacrifice of female rats. Relative liver weights of the HC 27-S treated male rats were increased over controls by 70, 175, and 180% at the 7, 21, and 35 days sacrifice periods, respectively, and those of the female treated rats were increased over controls by 35, 125, and 65% at the respective sacrifice periods. The relative liver weights of treated male rats continued to increase between sacrifice days 21 and 35 even though treatment with HC 27-S had been terminated. An increase ($p < 0.05$) in the relative testes weights of the treated male rats was noted at the 21- and 35-day sacrifice periods. However, the absolute weight of the testes increased at rates comparable to that of controls throughout the study.

Gross pathologic findings in rats at the conclusion of each treatment period consisted of enlarged livers, kidneys, and adrenal glands in both sexes. Enlarged mesenteric lymph

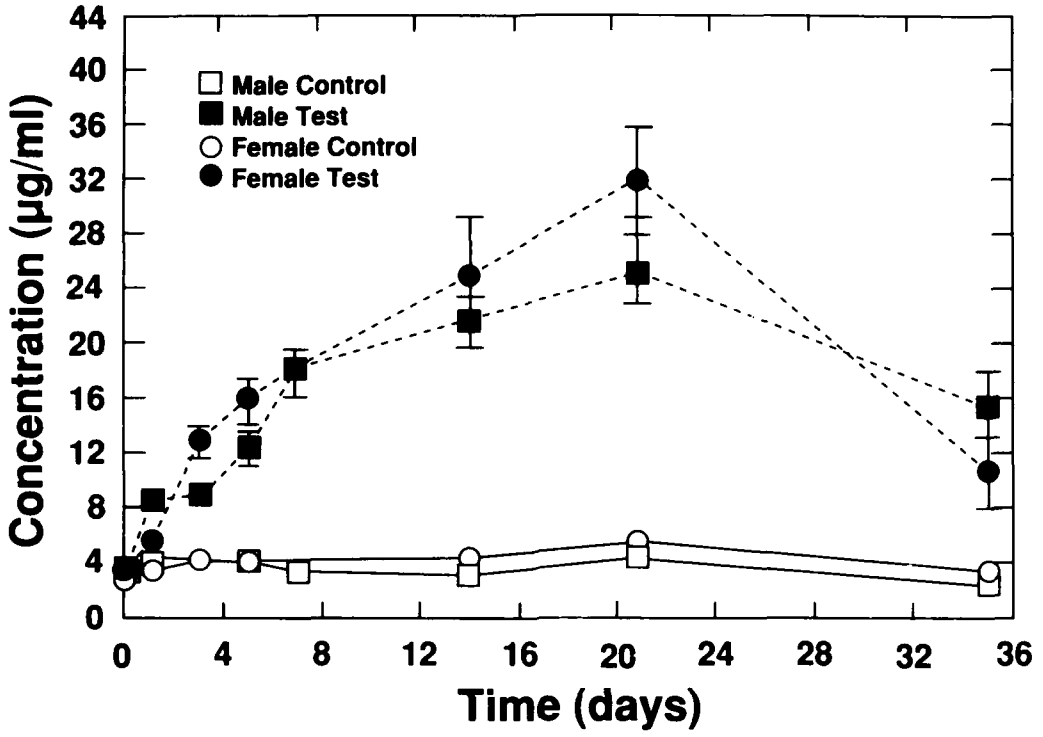

Figure 3. Mean urine fluoride concentration (± S.E.M.) for male and female Fischer-344 rats following repeated oral administration of Halocarbon 27-S for 21 days. N = 14 to 18 for days 1 through 7; 8 to 12 for days 8 through 21; and 4 to 6 for days 21 through 35.

nodes were noted in female rats killed after 7 days. Generalized atrophy of the thymus was noted in a number of the male rats killed at 21 and 35 days.

Microscopic changes were restricted to the organs of deglutition (pharynx/larynx and esophagus), mandibular lymph nodes, thoracic cavity, liver, adrenal glands, and possibly the kidneys. Most lesions in the organs of deglutition and thoracic cavity were compatible with gavage trauma. Moderate to severe acute/chronic inflammation and hemorrhage were observed in the larynx, esophagus, and thoracic cavity of rats of both sexes from all treatment groups. Mild to moderate hepatocellular swellings were present in 100% of the male rats treated with HC 27-S. Concurrently, 67%, 100%, and 83% of

TABLE 3
Plasma Fluoride Concentrations of Rats at Sacrifice Following Repeated Oral Administration of HC 27-S

	Fluoride Concentrations,[a] μg/mL		
Sex/Group	7 Days	21 Days	35 Days
Males			
Control	0.20 ± 0.03(6)	0.46 ± 0.08(5)	0.75 ± 0.05(3)
Test	0.16 ± 0.02(5)	0.56 ± 0.09(5)	0.45 ± 0.06(5)[b]
Females			
Control	0.29 ± 0.02(6)	0.31 ± 0.09(3)	0.37 ± 0.05(3)
Test	0.23 ± 0.03(6)	0.23 ± 0.01(3)	0.23 ± 0.01(5)[b]

[a] Mean ± S.E.M. (N).
[b] Statistically different from controls $p < 0.01$.

TABLE 4
Mean[a] CaP Measurements of Femurs from Rats Following Repeated Oral Administration of HC 27-S (N = 3)

Sex/Group	7 Days	21 Days	35 Days
Males			
Control	1.63 ± 0.02	1.64 ± 0.02	1.55 ± 0.03
Test	2.05 ± 0.02[b]	1.96 ± 0.02[b]	1.87 ± 0.06[b]
Females			
Control	1.63 ± 0.02	1.59 ± 0.01	1.68 ± 0.01
Test	1.66 ± 0.03	1.66 ± 0.02	1.68 ± 0.03

[a] Mean ± S.E.M.
[b] Statistically different from controls $p < 0.01$.

the treated females sacrificed following 7, 21, and 35 days, respectively, exhibited hepatocellular swelling.

Minimum to mild vacuolar degeneration (diagnosed as fatty change) of adrenocortical cells was present in 100% of the treated males and 50% of the treated females killed immediately following the 21-day treatment. Similarly, 67% and 83% of the males and females, respectively, killed following the 14-day recovery period also exhibited mild adrenocortical vacuolar changes. Adrenocortical changes were noted in only one control animal from the study.

Although increased weights were reported in kidneys harvested from treated rats, microscopic findings were generally unremarkable. Four of six treated male rats killed at 7 days displayed mild accumulations of hyaline droplets (resorbed protein) in the cyto-

TABLE 5
Mean[a] Organ Weights (g) and Organ to Body Weight Ratios[b] of Fischer-344 Rats Following Repeated Oral Administration of Halocarbon 27-S

		7 Days		21 Days		35 Days	
Sex	Observation	Control (6)	Test (5)	Control (6)	Test (5)	Control (6)	Test (5)
Male	Brain	1.77 ± .01	1.77 ± .02	1.77 ± .02	1.79 ± .00	1.91 ± .01	1.88 ± .01
	Ratio	0.87 ± .02	0.85 ± .01	0.80 ± .02	0.88 ± .01	0.74 ± .01	0.92 ± .03[c]
	Thymus	0.23 ± .01	0.23 ± .01	0.26 ± .01	0.19 ± .01[c]	0.29 ± .01	0.21 ± .02[d]
	Ratio	0.11 ± .01	0.11 ± .00	0.12 ± .00	0.09 ± .00	0.11 ± .01	0.10 ± .01
	Heart	0.73 ± .02	0.71 ± .01	0.81 ± .03	0.72 ± .02	0.97 ± .02	0.76 ± .03[c]
	Ratio	0.36 ± .01	0.34 ± .01	0.37 ± .01	0.35 ± .01	0.37 ± .01	0.37 ± .02
	Liver	6.60 ± .42	11.51 ± .33[c]	7.23 ± .41	17.90 ± .33[c]	8.08 ± .17	17.90 ± .69[c]
	Ratio	3.21 ± .15	5.53 ± .12[c]	3.25 ± .11	8.80 ± .21[c]	3.12 ± .04	8.73 ± .12[c]
	Kidney	1.64 ± .08	1.95 ± .04	1.78 ± .06	2.08 ± .02	2.07 ± .03	2.35 ± .10
	Ratio	0.80 ± .03	0.94 ± .02[c]	0.80 ± .01	1.02 ± .01[c]	0.80 ± .01	1.15 ± .02[c]
	Whole Body	204.5 ± 4.1	206.5 ± 2.0	222.2 ± 6.3	203.6 ± 3.1	259.3 ± 4.4	204.8 ± 6.5[c]
		Control (6)	Test (5)	Control (6)	Test (3)	Control (6)	Test (5)
Female	Brain	1.64 ± .02	1.66 ± .02[d]	1.64 ± .01	1.71 ± .00[d]	1.69 ± .02	1.71 ± .02[d]
	Ratio	1.20 ± .02	1.35 ± .06[d]	1.15 ± .03	1.19 ± .04	1.06 ± .02	1.08 ± .03
	Thymus	0.23 ± .01	0.21 ± .03	0.23 ± .01	0.22 ± .01	0.25 ± .02	0.25 ± .02
	Ratio	0.17 ± .01	0.17 ± .02	0.16 ± .01	0.15 ± .01	0.16 ± .01	0.16 ± .01
	Heart	0.56 ± .02	0.50 ± .03	0.52 ± .01	0.50 ± .01	0.63 ± .02	0.67 ± .02
	Ratio	0.41 ± .02	0.40 ± .02	0.37 ± .01	0.35 ± .02	0.40 ± .01	0.42 ± .01
	Liver	4.04 ± .12	5.16 ± .38	4.03 ± .10	8.96 ± .12[d]	4.66 ± .18	7.59 ± .47[d]
	Ratio	2.96 ± .05	4.14 ± .18[c]	2.81 ± .06	6.26 ± .29[c]	2.92 ± .07	4.81 ± .40[c]
	Kidney	1.05 ± .03	1.11 ± .04	1.10 ± .03	1.29 ± .01[d]	1.26 ± .04	1.47 ± .03[d]
	Ratio	0.77 ± .01	0.90 ± .03[c]	0.77 ± .01	0.90 ± .04[c]	0.79 ± .01	0.93 ± .04[c]
	Whole Body	136.7 ± 2.8	123.7 ± 5.4	143.3 ± 2.5	143.7 ± 5.5	159.3 ± 3.7	159.0 ± 4.8

[a] Mean ± S.E.M. (N).
[b] Organ weight/body weight × 100.
[c] Statistically different from controls at $p < 0.01$.
[d] Statistically different from controls at $p < 0.05$.

plasm of proximal tubular epithelial cells. Similar hyaline droplets were not recorded in males killed at the 21- or 35-day sacrifice. Relative testes weights were increased in male rats sacrificed at 21 and 35 days.

DISCUSSION

Depression of body weight gains throughout the first 21 days of the study appears to be related to treatment and housing (metabolism cages with ground food, a daily 16 hr fast period and a reversal in normal eating pattern), even though the rats had a one-week acclimatization period prior to this treatment. Following the 21-day treatment period the rats were returned to polycarbonate cages, pelletized food and unfasted conditions. All groups except the male test group showed a dramatic increase in mean body weights during the subsequent 14 days. In addition to the weight loss as the study progressed, the rats became increasingly irritable and difficult to handle, resulting in a number of cases of gavage trauma.

The elevated concentrations of serum BUN in the male rats indicate that renal clearance may have been affected. Return toward normal values was not apparent following the 14-day posttreatment period. The elevated serum albumin levels in both sexes of treated rats may be related to the reduced water consumption and resultant dehydration of the test rats.

The major route of fluoride excretion is by the kidneys (Haynes and Murad, 1985) and was obvious in this study as daily levels of inorganic fluoride in the urine of the test rats was significantly increased throughout the study. The mean concentration of inorganic fluoride in the urine of the test rats, examined following 14 and 21 days of treatment, ranged between 22 and 32 mg/l, approximately 6 to 7 times that of the respective controls. Fourteen days following cessation of treatment, the test rats were still excreting between 11 and 16 mg inorganic fluoride/l compared with control rats which excreted between 2 and 6 mg/l throughout the study. It is reported in Patty's Industrial Hygiene and Toxicology (1963) that the mean concentration of inorganic fluoride in the urine of Danish cryolite workers who complained of loss of appetite, shortness of breath and nausea was 16.05 mg/l (range of 2.41 to 43.41). In those workers with less severe exposure, the mean urinary concentration of fluoride was 4.81 mg/l (range of 1.78 to 11.67). It was also reported that heavily exposed aluminum workers (aluminum is produced by the electrolysis of bauxite in a bath of molten cryolite) had a mean daily urine fluoride concentration of 9.03 mg/l. In two factories in the United States, increases in the radiographic density of the bones have appeared in men whose urine was known to have contained 10 or more mg inorganic fluoride/l (Patty, 1963).

Plasma inorganic fluoride concentrations measured at 7, 21, and 35 days were not elevated when compared with controls. Previous acute studies with CTFE oligomers (Kinkead et al., 1987) reported significantly elevated plasma fluoride 7 days after a single oral dose. However, in this study the blood samples of the test animals were, at 5 of the

6 examination times, lower in fluoride concentrations than the control animals. The data suggests that repeated oral doses may have evolved an adaptive response which improved the kidney's ability to remove fluoride from plasma. However, an enhanced clearance mechanism as a result of repeated HC 27-S dosing was not confirmed. A report of a series of five-day balance studies in human subjects conducted several years ago disclosed that excretion exceeded intake in subjects given 1.5 to 6 mg of fluoride per day in food and water (McClure et al., 1945).

Previous investigators (Clayton et al., 1977; Potter et al., 1981; Buckley et al., 1982) reported increased water uptake and urine output in rats treated with or exposed to CTFE. The increases were accompanied by significant decreases in urinary osmolalities. Rats treated with HC 27-S exhibited the opposite effects with significant decreases in both water consumption and urine output and no appreciable change in urine concentrating ability.

The CaP ratios obtained in this study were from the mineralized matrix of rat femurs below the periosteum. This area of bone should reflect any changes in bone metabolism or mineralization due to its proximity to the periosteum, the area of rapid bone turnover. The CaP ratios were in the normal physiological range of 1.3 to 2.0 (Guyton, 1976) for all but one group of male rats. The ratio greater than 2.0 (2.05 $\pm$ 0.02 SEM) from the male group treated for 7 days appeared to peak at 7 days, then level out over the 21 days of treatment, and slowly decreased after cessation of treatment. Since bone turnover in rats is rapid, this is not surprising. In identically treated female rats, CaP ratios did not vary from control female CaP ratios.

The activity of serum alkaline phosphatase was elevated in the treated male rats at each sacrifice period. A slight increase in serum alkaline phosphatase activity in treated female rats was observed only at the final sacrifice. The most likely explanation for the higher enzyme activity in male rats is their more severe hepatocytomegalic liver disease. Enlarged hepatocytes probably compressed canaliculi and small biliary ducts causing partial intrahepatic cholestasis. Bile flow obstruction very commonly leads to the induction of alkaline phosphatase synthesis in the liver. Correspondingly, SGOT and SGPT, derived from injured hepatocytes, becomes elevated in the blood, as occurred in the Halocarbon 27-S treated male rats. The slightly higher serum alkaline phosphatase activities of male controls as compared to female controls could be reflective of the faster growth rate of the males as compared to females. Since fluoride is incorporated into the bone by replacing hydroxyapatite with the denser fluoroapatite (calcium fluoride, Haynes and Murad, 1985), it is reasonable to expect that the CaP ratio will increase as fluoride replaces phosphorus in the bone. Fluoride is preponderantly deposited in the skeleton and teeth, and the degree of skeletal storage is related to intake and age (Haynes and Murad, 1985). This is thought to be a function of the turnover rate of skeletal components, with growing bone showing a greater fluoride deposition than bone in mature animals. Although both male and female rats were identical in age during this study, male rats typically grow at a more rapid pace than females

(approximately 2 times) which may explain the change in CaP observed only in the male rats.

The description of thymus atrophy was a subjective judgment made during necropsy which was not verified by histopathologic examination. Inflammation and hemorrhage of the deglutitory organs can be attributed to the overall debilitated condition of the rats which resulted in increased difficulty performing the daily gavage treatment. The gavage trauma produced chronic esophagitis which resulted in lymphocytosis and hyperplastic mesentery and mandibular lymph nodes. Hepatocellular swelling was considered to be a distinct, treatment-related finding in this study and was persistent after the 14-day posttreatment period. The cytoplasmic changes may have included proliferation of smooth endoplasmic reticulum and/or peroxisomes. Studies designed to assess the ultrastructural bases for the hepatocytic lesions and to compare hepatic effects of HC 27-S with other compounds believed to cause similar hepatocytic effects are in progress. It was also apparent that adrenocortical vacuolar degeneration was a treatment-related effect which remained, unreversed, following the 14-day posttreatment period. Although increased kidney weights were noted in all treatment groups, no necrosis of the *pars recta* of the proximal tubules or degenerative changes were found as have been reported in rats exposed to CTFE monomers (Buckley et al., 1982).

In summary, this study indicated that repeated oral administration of HC 27-S results in acute toxic effects not unlike those of acute fluoride poisoning. Although HC 27-S is a mixture of polymers of CTFE, the overall toxic effects were not typical of those observed following intoxication with CTFE. Signs of nephrotoxicity, which would be expected in acute fluoride toxicity, diuresis, and increased water consumption, which are typical of CTFE toxicity, were not evident. Urine concentrating ability was unaffected in both sexes of rat. Defluorination, indicated by increased urine fluoride levels and changes in CaP ratios in the femurs of male rats, was evident throughout the study. Gross liver enlargement and microscopic hepatocytomegaly are findings which indicate that the liver is probably the primary rat organ injured by the repeated oral doses of HC 27-S used in this study.

REFERENCES

BARCIKOWSKI, R.S. (1983). Computer Packages and Research Design, Vol. 1: BMDP, University Press of America, Lanham, Maryland.

BUCKLEY, L.A., CLAYTON, J.W., NAGLE, R.B. and GANDOLFI, A.J. (1982). Chlorotrifluoroethylene Nephrotoxicity in Rats: A Subacute Study. Fund. Appl. Toxicol. **2**:181–186.

CLAYTON, J.W. (1977). Toxicology of Fluoroalkenes: Review and Research Needs. Environ. Health Perspect. **21**:255.

DIXON, W.J. (1985). BMDP Statistical Software. University of California Press, Berkeley, California.

GUYTON, A.C. (1976). Textbook of Medical Physiology, pp. 973–981. W.B. Saunders Company, Philadelphia, Pennsylvania.

HAYNES, R.C., JR. and MURAD, F. (1985). Agents Affecting Calcification: Calcium, Parathyroid Hormone, Calcitonin, Vitamin D, and Other Compounds. *In*: Goodman and Gilman's The Pharmacological Basis of Therapeutics. 7th Edition, pp. 1517–1540. Macmillan Publishing Company, New York, New York.

KINKEAD, E.R., GAWORSKI, C.L., HORTON, J.R. and BOOSINGER, T.R. (1987). Chlorotrifluoroethylene Oligomer: Evaluation of Acute Delayed Neurotoxicity in Hens, and Study of Absorption and Metabolism in Rats Following Oral, Dermal, and Inhalation Exposure. AAMRL-TR-87-044 (AD 187611), Harry G. Armstrong, Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio.

KINKEAD, E.R., CULPEPPER, B.T., HENRY, S.S., SZOTAK, P.S., FLEMMING, C.D., KUTZMAN, R.S., BRUNER, R.H., WYMAN, J.F. and MATTIE, D.R. (1989). The Determination of the Acute and Repeated Oral Toxicity of Halocarbon Oil, Series 27-S. AAMRL-TR-89-007, NMRI-88-16, Harry G. Armstrong Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio.

McCLURE, F.J., MITCHELL, H.H., HAMILTON, T.S. and KINSER, C.A. (1945). Balance of Fluoride Ingested from Various Sources in Food and Water by Five Young Men. Excretion through the Skin. J. Industrial Hygiene and Toxicology, **27**:159.

NEEFUS, J.D., CHOLAK, J. and SALTZMAN, B.E. (1970). The Determination of Fluoride in Urine Using a Fluoride-Specific Ion Electrode. Am. Ind. Hyg. Assoc. J. **31**:96–99.

PATTY, F.A., ed. (1963). Industrial Hygiene and Toxicology, pp. 835–841, 2nd Edition. Wiley and Sons, Inc., New York.

POTTER, C.L., GANDOLFI, A.J., NAGLE, R. and CLAYTON, J.W. (1981). Effects of Inhaled Chlorotrifluoroethylene and Hexafluoropropene on the Rat Kidney. Toxicol. Appl. Pharmacol. **59**:431–440.

SINGER, L. and OPHAUG, R.H. (1979). Concentrations of Ionic, Total, and Bound Fluoride in Plasma. Clin. Chem. **25**(4):523–525.

WIDMANN, F.K. (1973). Goodale's Clinical Interpretation of Laboratory Tests. 7th Ed. F.A. Davis Company, Philadelphia, Pennsylvania.

ZAR, J.H. (1974). Biostatistical Analysis. Prentice Hall, Englewood Cliffs, New Jersey.